Cortical and Cancellous Adaptation of Mouse Tibiae to In Vivo Mechanical Loading is a Purely Local Response

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INTRODUCTION:

Different *in vivo* models demonstrate that dynamic loading of the skeleton is osteogenic (1,2). Previously, our group has shown that compressive *in vivo* loading of one tibia in 10-week-old mice increases bone mass and alters bone architecture when compared to the contralateral limb (1,3). These effects of unilateral applied loads are often assumed to be local responses as demonstrated in a mouse tibial loading model (4). Alternatively, neuronally-driven systemic effects in rats have been suggested (5). For our tibial loading model, whether compressive loading in one tibia affects the opposing tibia or alters systemic hormone levels has not been validated. This study aims to validate that the osteogenic response seen in cortical and cancellous bone in our tibial compression model is purely a local phenomenon.

METHODS:

Twenty 10-week-old female C57Bl/6 mice were divided into two groups: Loaded and Sham (n=10 per group). Loaded mice were anesthetized (2% isoflurane, 1L per min), and the left tibia of each mouse was subjected to a dynamic compressive *in vivo* load, 5 days a week for 2 weeks, at 4Hz for 1200 cycles/day. The triangle loading waveform had a peak load of 9N, which previously has been shown to be osteogenic in murine tibiae (6). Sham mice were anesthetized and the left limb was fixed in the custom loading device for the duration of 1200 cycles (5 minutes) but was not loaded. The right limbs of both groups were not fixed in the device or loaded. After two weeks, animals were euthanized by cardiac puncture while under anesthesia, and blood was collected and clotted to obtain serum. All experimental procedures were performed under an IACUC-approved protocol.

Left and right tibiae were scanned by microcomputed tomography at a resolution of 15 µm (µCT35, Scanco Medical AG). For each tibia, cortical and cancellous regions of interest were analyzed. The cortical region of interest was defined as 2.5% of tibial length centered around the midshaft. Cortical outcomes were area (Ct.Ar, mm^2), tissue mineral density (ctTMD, mg HA/cm^3), minimum moment of inertia (I_{MIN}, mm^4), and maximum moment of inertia (I_{MAX}, mm^4). The cancellous region of interest in the proximal tibia began immediately distal to the primary spongiosa and extended 10% of total tibial length. Cancellous outcomes were bone volume fraction (BV/TV), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), and tissue mineral density (cnTMD, mg HA/cm^3).

In addition, serum ELISAs were performed in triplicate for each mouse to determine estrogen (E2) and testosterone (T) levels in the Loaded and Sham mice (Endocrine Technologies, ERK-R7016 and ERK-R7005).

The cortical and cancellous responses to the two experimental protocols (Loaded vs. Sham) were determined using a linear mixed-model with repeated-measures and a post-hoc Bonferroni correction (SPSS, v.18). The between-subject factor was experimental protocol (Loaded versus Sham) and the within-subject factor was limb (left versus right tibia). Student's t-tests were used to determine if serum E2 and T levels were different between Loaded and Sham animals. Significance was set at p-values less than 0.05. Values are reported as mean ± standard deviation.

RESULTS:

The left tibiae of the Loaded animals showed adaptive responses to mechanical loading in both cortical and cancellous bone (Table 1). Compared to the right limb, the loaded tibiae had 59% larger I_{MAX} and 36% larger I_{MIN}. In addition, Ct.Ar was increased in the left limb by 32% compared to the right limb (Figure 1A). CtTMD was not affected by loading. In the cancellous compartment, the left tibiae showed 37% increased BV/TV (Figure 1B), mostly due to 35% increased Tb.Th. CnTMD increased 8%.

The right control tibiae from the Loaded animals were not significantly different from the left and right limbs of the Sham animals for any measurable outcomes.

Serum levels of estrogen and testosterone were not significantly different between the Loaded and Sham animals, suggesting that unilateral hind limb loading did not have a systemic effect on hormone levels (Table 2).

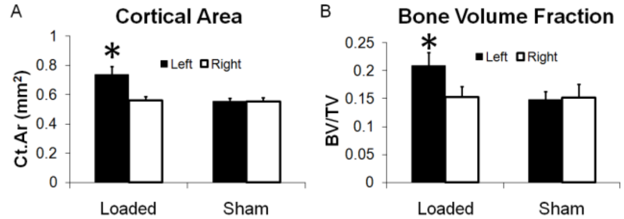

Figure 1. Ct.Ar and BV/TV for Loaded and Sham animals
* significant difference compared to right Loaded, left Sham, and right Sham

Table 1. Cortical and cancellous measurements for Loaded and Sham tibiae

Outcome Measure	Loaded		Sham	
	Left	Right	Left	Right
Ct.Ar (mm^2)	**0.74 ± 0.06***	0.56 ± 0.03	0.56 ± 0.02	0.55 ± 0.03
I_{MAX} (mm^4)	**0.098 ± 0.01***	0.062 ± 0.009	0.061 ± 0.005	0.058 ± 0.006
I_{MIN} (mm^4)	**0.067 ± 0.007***	0.049 ± 0.005	0.047 ± 0.004	0.047± 0.005
ctTMD (mg HA/cm^3)	923.9 ± 13.8	931.3 ± 9.1	925.5 ± 13	929.4 ± 14
BV/TV	**0.21 ± 0.023***	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
Tb.Th (µm)	**80.8 ± 3.3***	59.8 ± 2.8	58.0 ± 1.7	58.6 ± 2.4
Tb.Sp (µm)	248 ± 21	246 ± 19	246 ± 29	240 ± 26
cnTMD (mg HA/cm^3)	**626.1 ± 12***	580.4 ± 8	574.1 ± 16	573.2 ± 10

* significant difference compared to right Loaded, left Sham, and right Sham

Table 2. Serum hormone levels for Loaded and Sham animals

Serum Hormone	Loaded	Sham
Estrogen (pg/mL)	6.75 ± 1.21	6.37 ± 3.86
Testosterone (ng/mL)	54.4 ± 4.4	55.4 ± 4.1

DISCUSSION:

The use of contralateral control limbs to study the skeleton's response to mechanical loads is commonplace in skeletal adaptation studies. However, dynamic loading may induce systemic effects as previously reported in rats (5). The rats were divided into groups, where the right ulna was loaded cyclically, loaded to fatigue, or not loaded (5). In the loaded animals, periosteal area as labeled by confocal microscopy increased in six non-loaded bones, and TRAP5b serum levels increased compared to sham animals. These effects diminished when loaded animals underwent neuronal blockage, indicating a systemic origin. To validate our tibial compression model, we sought to ensure that bone adaptation is purely local. We found that the adaptive response of the tibia to mechanical loading was confined to the loaded limb. Also, systemic estrogen and testosterone levels were not different from baseline. However, other hormones that could affect skeletal remodeling were not examined in this study. These findings are similar to another mouse tibial loading model, which also found a purely local response as indicated by no significant differences among multiple microCT measurements of loaded and sham animals (4).

Through our two groups of mice, we demonstrated that the right non-loaded limb from the Loaded animals showed similar values for all parameters to both the left and right limbs of the Sham animals and was significantly different from the left loaded limb of the Loaded animals. Systemically, hormone levels were not altered by loading. These results show that the anabolic bone response to tibial mechanical loading in mice is purely local, which validates future use of this model in studying local bone adaptation to mechanical forces.

ACKNOWLEDGEMENTS:

We thank our funding sources: NIH R01-AG028664, NIH P30-AR046121, GAANN, NSF GRFP, NIH F32 AR054676, NSF LSAMP 06-522. We also thank Cornell's CARE staff for their assistance.

REFERENCES:

[1] Moustafa, *et al.*, Bone 2009. [2] Fritton, *et al.*, Bone 2005. [3] Lynch, *et al.*, J Appl Physiol 2010. [4] Sugiyama, *et al.*, Bone 2010. [5] Sample, *et al.*, JBMR 2010. [6] Main, *et al.*, ASMBR 2009.